

Applicants: Gloria C. Li et al.
Serial No.: 09/750,410
Filed : December 28, 2000
Page 2

In addition, please amend the specification under the provisions of 37 C.F.R. §1.121(b)(1) as follows.

On page 12, lines 10-23, please delete the paragraph which begins "Figure 14" and insert the following paragraph:

Figure 14

Nucleotide sequences of V β 8D β 2.1J β 2.6 junctions from the thymus of a 4 week old *Ku70*^{-/-} mouse (SEQ ID NOS: 1-23). Products corresponding to V β 8.1, V β 8.2 or V β 8.3 rearrangement with J β 2.6 were cloned and sequenced. TCR V β 8-J β 2 joints were amplified by PCR (20, 27, 28) as described (see Fig. 3B). PCR cycling conditions were 94°C for 45", 68°C for 30", and 72°C for 30" (30 cycles). The band corresponding to V β 8-J β 2.6 was purified, reamplified for 20 cycles and then subcloned into the pCRII vector (Invitrogen). DNA was extracted from individual colonies and sequenced using the universal T7 and M13 reverse primers. Germline sequences are written in bold case, 'N' and 'P' denote nucleotides not present in the germline sequences.

On page 24, lines 25-32, please delete the text which begins "The genotype of the mice was first determined . . ." and insert the following text:

The genotype of the mice was first determined by tail PCR analysis which distinguishes endogenous from the targeted *Ku70* allele, and subsequently confirmed by Southern blot analysis. The PCR reaction contained 1 μ g genomic DNA; 0.6 μ M (each) of primers HO-2: GGGCCAGCTCATTCCTCCACTCATG (SEQ ID NO: 24), HO-3: CCTACAGTGTACCCGGACCTATGCC (SEQ ID NO: 25) and HO-4: CGGAACAGGACTG-GTGGTTGAGCC (SEQ ID NO: 26); 0.2 mM (each) dNTP;

Applicants: Gloria C. Li et al.
Serial No.: 09/750,410
Filed : December 28, 2000
Page 3

1.5 mM MgCl₂ and 2.5 U of Taq polymerase. Cycling conditions were 94°C for 1 min,

On page 25, lines 8-20, please delete the paragraph which begins "To confirm that the disruption of *Ku70* . . ." and insert the following paragraph:

To confirm that the disruption of *Ku70* produces a null mutation, *Ku70* protein expression was measured by Western blotting using polyclonal antisera against intact mouse *Ku70*. The lack of *Ku70* was also verified by a Ku-DNA-end binding assay (gel mobility shift analysis). Cell extracts were prepared and gel mobility shift assays were performed as described (22). Equal amounts of cellular protein (50 µg) from *Ku70*^{+/+} (WT), *Ku70*^{+/-}, and *Ku70*^{-/-} mouse embryo fibroblasts were incubated with a ³²P-labeled double-stranded oligonucleotide, 5'-GGGCCAAGAATCTTCCAGCAGTTTCGGG-3' (SEQ ID NO: 27). The protein-bound and free oligonucleotides were electrophoretically separated on a 4.5% native polyacrylamide gel. Gel slabs are dried and autoradiographed with Kodak X-Omat film.

On page 27, lines 1-23, please delete the paragraph which begins "To determine whether a null mutation . . ." and insert the following paragraph:

To determine whether a null mutation in *Ku70* affects the recombination of antigen-receptor genes in T and B lymphocytes *in vivo*, we measured the immunoglobulin and T-cell antigen receptor (TCR) rearrangements by PCR. DNA from bone marrow was amplified with primers specific to immunoglobulin D-J_H and V-DJ_H rearrangements, and DNA from thymus was amplified with primers that detect V-DJ_β and D_δ-J_δ-rearrangement (20, 25-28).

Oligonucleotides for probes and PCR primers specific to TCR V β -J β rearrangements and immunoglobulin D-J $_H$ and V-DJ $_H$ rearrangements are as follows. For TCR β V β 8-J β 2 rearrangements (28): V β 8.1: 5'-GAGGAAAGGT-GACATTGAGC-3' (SEQ ID NO: 28), J β 2.6: 5'-GCCTGGTGCCGGGACCGAAGTA-3' (SEQ ID NO: 29), V β 8 probe: 5'-GGGCTG AGGCTG ATCCATTA-3' (SEQ ID NO: 30). For D δ 2-J δ 1 rearrangement (20, 27): DR6: 5'-TGGCTTGACATGCAGAAAACACCTG-3' (SEQ ID NO: 31), DR53: 5'-TGAATTCCACAG-TCACTTGGCTTC-3' (SEQ ID NO: 32), and DR2 probe: 5'-GACACGTGATACAAAGCCCAGGGAA-3' (SEQ ID NO: 33). For immunoglobulin D-J $_H$ and V-DJ $_H$ rearrangements (26): 5'D: 5'-GTCAAGGGATCTACTACTGTG-3' (SEQ ID NO: 34), V7183: 5'-GAGAGAATTCAGAGACAATC-CCAAGAACACCCTG-3' (SEQ ID NO: 35), VJ558L: 5'-GAGAGAATTCTCCTCCAGCACAG-CCTACATG-3' (SEQ ID NO: 36), J2: 5'-GAGAGAATTCGGCTCCCAATGACCCTTTCTG-3' (SEQ ID NO: 37), 5'IVS: 5'-GTAAGAATGGCCTCTCCAGGT-3' (SEQ ID NO: 38), 3'-IVS: 5'-GACTCAATCACTAAGACA-GCT-3' (SEQ ID NO: 39), and probe: a 6 kb EcoR I fragment covering the J region of mouse IgM.

On page 62, lines 23-34, please delete the text which begins "The genotypes of the mice were first determined . . ." and insert the following text:

The genotypes of the mice were first determined by tail PCR analysis which distinguishes endogenous from the targeted Ku70 allele, and subsequently confirmed by Southern blot analysis. The PCR reaction contained 1 mg genomic DNA; 0.6 mM (each) of primers HO-2: GGGCCAGCTCATTCCTCCACTCATG (SEQ ID NO: 40), HO-3: CCTACAGTGTACCCGGACCTATGCC (SEQ ID NO: 25) and HO-4: CGGAACAGGACTGGTGGTTGAGCC (SEQ ID NO: 41); 0.2 mM (each) dNTP; 1.5 mM MgCl₂ and 2.5 U of Taq polymerase. Cycling conditions were 94°C for 1 min, 64°C for 1 min, 72°C for 1 min (30 cycles), followed by an extension at 72°C for 10 min. Primers HO-2 and HO-4 give a product of the targeted

Applicants: Gloria C. Li et al.
Serial No.: 09/750,410
Filed : December 28, 2000
Page 5

On page 87, lines 17-25, please delete the paragraph which begins "The genotype of the mice was determined by PCR . . ." and insert the following paragraph:

The genotype of the mice was determined by PCR which distinguishes endogenous from the targeted DNA-PKcs allele. PCR reaction contains 1 μ g genomic DNA; 0.6 μ M (each) of primers MD-20: TATCCGGAAGTCGCTTAGCA-TTG (SEQ ID NO: 42); MD-21: AAGACGGTTGAAGTCAGAAGTCC (SEQ ID NO: 43); and POL-8: TTCACATACACC-TTGTCTCCGACG (SEQ ID NO: 44); 0.2 mM(each) dNTP; 1.5 mM $MgCl_2$ and 2.5U of Taq polymerase. Primers MD-20 and MD-21 give a product of wild type allele that is 264 bp; primers MD-20 and Pol-8 yield a product of the targeted allele that is 360 bp.

On page 88, lines 7-19, please delete the paragraph which begins "For RT-PCR assay, total RNA was prepared . . ." and insert the following paragraph:

For RT-PCR assay, total RNA was prepared from SV40 transformed lung fibroblast cells using Qiagen RNeasy kit (Qiagen Inc., Santa Clarita, CA). After digestion of contaminated genomic DNA by DNase I (Ambion, Austin TX), cDNA synthesis was carried out with the Superscript preamplification system (Gibco BRL, Gaithersburg, MD) according to the included protocol. PCR primers used for RT-PCR were MD-3: ATCAGAAGGTCTAAGGCTGGAAT (SEQ ID NO: 45), MD-5: CGTACGGTGTGGCTACTGC (SEQ ID NO: 46) for amplification between exon 1 and 4 of DNA-PKcs, MD-28: CACTGAGGGCTT-TCCGCTCTTGT (SEQ ID NO: 47), MD-29: GCTCTTGTGCACGAATGTTGTAG (SEQ ID NO: 48) for PI-3 kinase domain, and GA-5: AGAAGACTGTGGATGGCCCC (SEQ ID NO: 49), GA-3: AGGTCCACCACCC-TGTTGC (SEQ ID NO: 50) for control GAPDH amplification.

Applicants: Gloria C. Li et al.
Serial No.: 09/750,410
Filed : December 28, 2000
Page 6

On page 89, line 22 - page 90, line 12, please delete the paragraph which begins "T cell antigen receptor (TCR) . . ." and insert the following paragraph:

T cell antigen receptor (TCR) and immunoglobulin recombination in T and B lymphocytes were measured by amplifying rearranged DNA fragments using PCR. Genomic DNAs were isolated from thymus, spleen and bone marrow (BM) from 4-to 9-week-old DNA-PKcs heterozygous (+/-), homozygous (-/-) mice and SCID mice. Oligonucleotides for PCR primers and probes are as follow. For TCR β V β 8-J β 2 rearrangement (16), V β 8.1: GAGGAAAGGTGACATTGAGC (SEQ ID NO: 51), J β 2.6: GCCTGGTGCCGGGACCGAAGTA (SEQ ID NO: 29), and V β 8 probe: GGGCTGAGGCTGATCCATTA (SEQ ID NO: 52). For TCR δ D δ 2-J δ 1 rearrangement, DR6: TGGCTTGACATGCAGAAAACACCTG (SEQ ID NO: 31), DR53: TGAATTCCACAGTCACTTGGGTTC (SEQ ID NO: 53) and DR2 probe: GACACGTGATACAAAGCCCAGGGAA (SEQ ID NO: 33). For TCR δ D δ 2-J δ 1 signal joint (19), DR21: GTCATATCTTGTCCAGTCAACTTCC (SEQ ID NO: 54), DR162: GATGAGCCAGCTGGATGAGTAACAC (SEQ ID NO: 55), and DR161 probe: GCCCTCTAGCCATGACA TCAGAGC (SEQ ID NO: 56). For immunoglobulin V H 7183-J H 4 rearrangement (19), DR214: CGCGAAGCTTCGT GGAGTCTGGGGGA (SEQ ID NO: 57), DR217: GGGGAATTCCTGAGGAGACGGTGA CT (SEQ ID NO: 58), and DR218 probe: ACCCCAGTAGTCCATAGCATAGTAAT (SEQ ID NO: 59). For control GAPDH amplification, same primers were used as RT-PCR experiment. Probe DNA for mouse GAPDH was purchased from Ambion Inc.(Cat.#7330, Austin TX). Amplified PCR products were resolved on 2% of agarose gel in 0.5x TBE, and transferred to Hybond N+ nylon membrane. Using radiolabeled oligonucleotide or DNA probes, PCR products were hybridized and visualized by autoradiography.